Discovery of Multiple Lead Compounds as M2 Inhibitors through the Focused Screening of a Small Primary Amine Library

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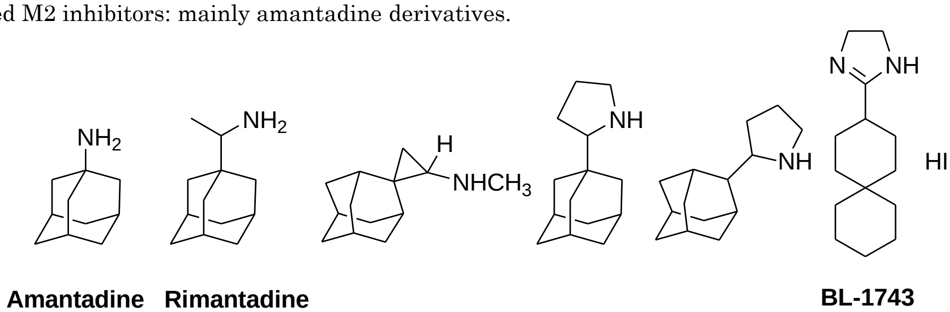
Abstact: The discovery of new anti-influenza drugs is urgent, particularly considering the recent threat of swine flu. Although amantadine derivatives are the only M2 drugs for influenza virus A, their use is limited in the US due to drug resistance. Here we report the discovery of multiple M2 inhibitor lead compounds that were rapidly generated through focused screening of a small primary amine library which was designed using a scaffold-hopping strategy based on amantadine.

There is currently an outbreak of H1N1 influenza (swine flu) around the world[1,2]. Although vaccination is the ideal way to prevent influenza virus infection, the preparation of a new vaccine requires more than 6 months[3]. Thus, antiviral drugs are most effective for short-term defense against influenza. However, very few effective drugs are available to combat the influenza virus.

The only known anti-influenza A drugs[3-6] are M2 inhibitors (amantadine and its derivative rimantadine) and NA inhibitors (zanamivir and oseltamivir). Amantadine and rimantadine are limited in their use in the US due to the rapid development of resistance. In addition, there is growing concern that anti-neuraminidase-resistant viruses may emerge if these drugs are widely used[7]. Thus, there is an urgent need to discover new types of M2 inhibitors for the development of new anti-influenza drugs. Although amantadine reached the market 40 years ago, all known M2 inhibitors to date are amantadine derivatives (Figure 1), with the exception of BL-1743[8]. Therefore, a vast area of chemical space remains to be explored.

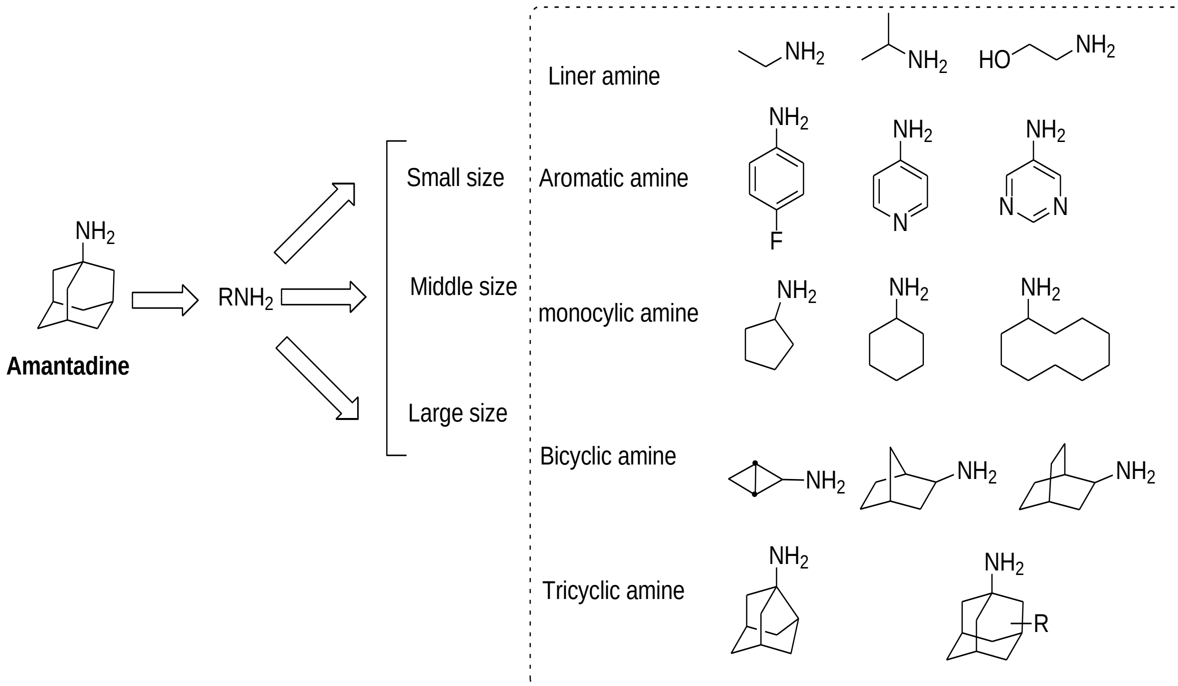
Many years of high-throughput screening (HTS) of various chemical libraries have not fulfilled expectations[9]. Although this strategy still plays a key role in lead generation, there is a growing interest in library design and analysis[10]. Focused screening has emerged as a more rational approach that emphasizes the quality rather than the quantity[11]. Dr. Gillet mentioned in his paper that[10] focused screening involves the selection of a subset of compounds according to existing structure-activity relationships. Although many publications[12-15] have discussed the trends and HTS application in drug discovery, there is a shortage of successful case studies that validate this approach.

Figure 1. Reported M2 inhibitors: mainly amantadine derivatives.



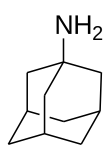
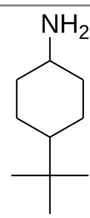
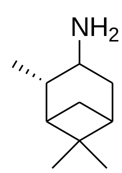
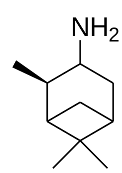
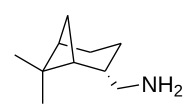
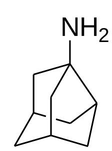
We decided to design and screen a small primary amine library of scaffold-hops[11] based on amantadine to generate new lead compounds in the M2 inhibitor class. Five compounds were identified as M2 inhibitors out of a library consisting of 70 molecules. Here, we demonstrate that focused screening is highly efficient in lead generation, and we describe the identification of multiple M2 inhibitors that may support anti-viral drug discovery.

Figure 2. The design strategy used for our primary amine library.



The mechanism of M2 inhibitors is to block the ion channel activity of the M2 protein of most influenza A viruses[16]. This action inhibits viral replication by blocking hydrogen ion flow. The amino group in amantadine is likely the pharmacophore and is necessary to block hydrogen ion transport. Consequently, the adamantyl group is the scaffold. For unknown reasons, nearly all studies except for those investigating BL-1743 have focused on the search for new aminoadamantane derivatives[17], with much less attention focused on the chemotype.

Table 1. Compounds that share the inhibitory activity of Amantadine identified in the small library.

No.	Amantadine	ZSG-2-101B	LSR-2-007C	LSR-2-007D	ZSG-2-046C	ZSG-2-101E
Structure						
Viral inhibition	7.447	33.49	6.018	1.363	2.304	38.21
Cell-based assay	3.525	3.37	22.98	5.960	25.47	18.97
Patch-clamp recording	8.8±2.7	4.8± 1.2	6.8±2.2	4.3±2.7	4.4±1.3	13.5± 4.1

IC_{50} (mean ± SEM) μM.

The strategy for our library design was simple and based on the structure and activity relationships of amantadine. The scaffold covers different molecular properties, with an emphasis on steric effect. As shown in Figure 2 and Table S1, this library contains linear, aromatic, monocylic, bicyclic, and tricyclic amines supplied by the major chemical companies. We rapidly constructed the library by ordering 70 primary amines from commercial sources.

The library was screened by employing three types of *in vitro* assays, including viral inhibition, a cell based assay, and patch clamp analyses. Among these 70 compounds, we found five compounds (Table 1) that could act as M2 inhibitors. Only compound ZSG-2-101E was less active than amantadine, and the other 4 compounds were more or less active in different assay models. Thus, these compounds were M2 inhibitors consisting of new chemotypes.

The five structures in Table 1 represent the extent of the middle steric effects. Compound ZSG-2-101B is a substituted cyclohexyl amine, LSR-2-007C and LSR-2-007D are bi-cyclic compounds and entio-isomers configured in R and S-methyl group, ZSG-2-046C has the same scaffold as the former compounds with a methyl amine as a functional group, and ZSG-2-101E is closely related to Amantadine but contains one less methylene. The structure and activity data suggest that the wild type M2 ion channel can accommodate a range of chemical space, but a minimal functional group is

required to block the channel. Whereas all linear, simple monocylic, and aromatic amines have no activity, substituted cyclohexylmaine, as well as some bicyclic and tricyclic amines, have inhibitory activities that mimic amantadine. Expansion of the size of Amantadine by the addition of substitute groups to the ring, such as methyl or hydroxyl groups, enhances inhibitory activity.

Although medicinal chemists typically do not use such alkyl scaffolds for drug development, risk-versus-benefit equations suggest the usefulness of these scaffolds. These newly discovered chemotypes may be used for further drug discovery targeted against acute and deadly infectious diseases. Thus, this study only validates that focused screening is practical for lead generation. In addition, we disclose several new M2 inhibitors for the discovery of new anti-influenza drugs. Thus, drug discovery in academia may benefit from the use of targeted library design and analysis rather than expensive HTS.

Supporting Information Available: Supporting Information is available free of charge via the Internet at http://pubs.acs.org.

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Synopsis TOC